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Alternate Approach

Mammalian Cell DNA Repair (J. D. Regan)

Our primary objective is to elucidate the molecular events in human cellular DNA damaged by radiation or chemical agents. We study and characterize: (a) the sequence of DNA repair events; (b) the various modalities of repair; (c) the genetic inhibition of repair due to mutation; and (d) the physiological inhibition of repair due to biochemical inhibitors.

Our technological capacities reside in the several experimental techniques we have developed for the assay for DNA repair. Examples are: (a) bromodeoxyuridine photolysis; (b) radiochromatography; (c) molecular weight analyses; and (d) enzymatic assays for DNA lesions. Our ultimate goals are: (a) to isolate and analyze the repair component of the mutagenic and/or carcinogenic event in human cells; and (b) to elucidate the significance of this repair component as it impinges on the practical problems of exposure to radiation or chemical mutagens and carcinogens.

Prereplication DNA Repair Modes Induced in Human Cells by Chemical Carcinogens

Damage to DNA incurred as a result of exposure to chemical agents is of great interest due to the correlation of such damage with mutagenesis and carcinogenesis. DNA repair of these lesions can be measured and yields information regarding the nature of the lesion, the physiological response of the cell and in some cases, the ability of the cell to activate a procarcinogen or mutagen. The bromodeoxyuridine (dBrU) photolysis assay can be used to categorize chemical agents as eliciting long-patch (UV-type) or short-patch (ionizing-irradiation type) excision repair, thus, further classifying the type of damage and response of the cell to the lesion

induced by the agent.

During the past years, this laboratory has obtained from other laboratories, both internally and externally, chemicals which are suspected carcinogens. This work relationship with other laboratories continues. We have found that a wide variety of known and suspected mutagens and carcinogens can elicit a DNA repair response from human fibroblasts.

Cell Culture

Experimental and control cells are obtained from reliable suppliers, such as the American Type Culture Collection, or initiated in our laboratory. Most of the cells needed are already stored in our liquid-nitrogen cell bank which houses more than 200 different cell lines. We routinely initiate additional primary cultures relevant to our work as biopsies become available. Cells are routinely checked for mycoplasms and other contaminations.

The cells are grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum and nonessential amino acids at 37°C in a humidified atmosphere at 2.5% CO_2 and air. Occasionally growth medium alterations are necessary for optimum growth of unusual cells.

For experiments, the cells are counted using an electronic laser-beam counter, planted in plastic petri dishes, incubated for 48 hr in growth medium, and labeled by incubating them for about 20 hr in medium containing radioactive DNA precursors. Cells for bromodeoxyuridine photolysis assays are labeled with ^{3}H-thymidine (1.9 Ci/mmole) at a concentration of 4 μCi/ml and ^{14}C-thymidine (500 mCi/mmole) at a concentration of 0.5 μCi/ml; cells for dimer analysis are labeled with ^{3}H-thymidine (50 Ci/mmole) at a concentration of 0.1 μCi/ml and ^{14}C-uridine (58 Ci/mmole) at a concentration

of 1 μCi/ml. After the labeling period, cells are incubated in nonradioactive growth medium for 2-3 hr.

Chemical Treatment of Cells

Chemical treatment of cells is handled in one of two methods generally. The cell, after labeling, is treated with a chemical in a sublethal dose for a given period of time. The chemical is removed and then either the DNA is analyzed for damage or the cell is given a period of time (usually 20-24 hr) to repair the DNA before analysis. The second method has the cell irradiated with 254 nm UV and then treated with a chemical to determine whether the chemical affects the repair or rate of repair of the DNA. UV irradiation is carried out by use of a single germicidal lamp (15T8, General Electric).

The dBrU Photolysis Assay

The usual procedure for this assay utilizes cells which have been prelabeled with 0.5 μCi/ml [^{14}C]thymidine (500 mCi/mmol) or 4 μCi/ml [Me-^{3}H]thymidine (1.9 Ci/mmol). Prior to any insult, the media-containing radioactive label is replaced with original growth media and incubated for 2 hr. Two dishes of cells are needed for each assay, one labeled with ^{14}C and one with ^{3}H as above. Immediately following treatment of both dishes with the insult (UV light, gamma rays, chemical agents, etc.), thymidine and dBrU (10^{-4} m) are added to the ^{14}C- and ^{3}H-labeled cells, respectively. These cells are then incubated for the chosen repair period, usually 18-20 hr, thus, allowing the incorporation of dBrU in the repair patch of the ^{3}H-labeled DNA. The cells are then harvested and mixed at a concentration of 2×10^{5} cells/ml. An aliquot of this cell mixture is placed in a quartz cuvette and given a series of 313 nm radiation doses from a Hilger quartz prism monochromator with a 1000-watt high pressure mercury arc source. This radiation sensitizes the dBrU-containing patches to alkali resulting in the appearance of single-strand breaks on alkaline

sucrose gradients. About 10^4 cells from each dose of 313 nm radiation are analyzed for single-strand breaks on alkaline sucrose gradients as previously described. The gradients are fractionated on filter paper strips which are then washed and counted for double label in a liquid scintillation system. The resulting data are analyzed by a PDP-11 computer which plots the radioactivity profiles and calculates the molecular weights of the ^{14}C- and ^{3}H-labeled DNAs. The difference of the reciprocal molecular weights ($\Delta 1/M_w$) are plotted as a function of the 313 nm light fluence. The number of resulting breaks for any given fluence of 313 nm can be calculated as 2 x $\Delta 1/M_w$ for that fluence. The total number of repaired sites detected can be estimated as 2 x $\Delta 1/M_w$ at 313 nm light saturation.

Given the following:

N = total number of repaired regions/10^8 daltons;

n = number of dBrU residues per region;

F = 313 nm light fluence in ergs/mm^2;

B = number of breaks or repaired regions for a given 313 nm fluence/10^8 daltons;

= 7.6×10^{-8} mm^2/erg = constant related to DNA cross section calculated from data on fully substituted DNA.

The probability of 313 nm radiation making a break in a given repaired region is: $1 - e^{-n\alpha F}$. If n is large such as in long-patch excision repair, then 313 nm light saturation will be reached; N can be estimated and the patch size estimated through the calculation of n in: $\frac{dB}{dF} = N n \alpha$.

Handling and Disposal of Chemicals and Radioactive Labels

All chemicals to be studied are stored in double-contained vessels and are opened and transferred in an exhaust hood enclosed glove box. Weight determinations are made in closed, pre-weighed vials. Only the amounts

necessary for experiments are made up in working solutions. Transfers of chemical-containing culture medium are performed under a laminar-flow hood equipped with HEPA particle filters. Disposable pipets and culture vessels are employed where chemical contact is anticipated. All such materials are disposed of in accordance with OSHA and UCC-ND guidelines.

Low-level radioactive precursors containing ^{3}H and ^{14}C are handled in disposable syringes, pipets and tissue culture vessels. All culturing materials and liquids contaminated by these precursors during experiments are disposed of according to plant regulations.